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6. AUTHORS Xiaoyang Zhu			5d. PROJECT NUMBER		
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			5f. WORK UNIT NUMBER		
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14. ABSTRACT This research program aims to develop block copolymer thin films as model systems for understanding protein activity in the immobilized state and as effective technologies to achieve optimal protein activity on surfaces. Protein molecules immobilized on solid surfaces underpin a number of key technologies in bio-analysis, such as ELISA, biosensors, and protein microarrays for the large-scale screening and profiling in proteomics. A solid surface is intrinsically a foreign environment to protein molecules but little is known about the relationship between					
15. SUBJECT TERMS block copolymer, protein immobilization, polymer coating					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON Xiaoyang Zhu
a. REPORT UU	b. ABSTRACT UU	c. THIS PAGE UU			19b. TELEPHONE NUMBER 612-624-7849

## Report Title

### Controlling Protein Conformation & Activities on Block-Copolymer Nanopatterns

#### ABSTRACT

This research program aims to develop block copolymer thin films as model systems for understanding protein activity in the immobilized state and as effective technologies to achieve optimal protein activity on surfaces. Protein molecules immobilized on solid surfaces underpin a number of key technologies in bio-analysis, such as ELISA, biosensors, and protein microarrays for the large-scale screening and profiling in proteomics. A solid surface is intrinsically a foreign environment to protein molecules but little is known about the relationship between surface chemistry and protein conformation/activity in the immobilized state. The long-term goal of this project is to develop fundamental understanding of the essential structure-property relationship between the chemical structure of the surface and the activity of immobilized protein molecules using block copolymer thin films to create chemical and geometrical patterns for the selective immobilization of protein molecules. During the past year, we successfully developed a number of block copolymer coatings, particularly PS-PHEMA and PS-PMMA in the cylinder phases. We carried out surface chemical modifications of the cylinder domains and immobilized poly-His tagged protein molecules in a spatially selective manner. We also succeeded in quantifying protein adsorption kinetics on these patterned surfaces.

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#### List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

##### (a) Papers published in peer-reviewed journals (N/A for none)

Number of Papers published in peer-reviewed journals: 0.00

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##### (b) Papers published in non-peer-reviewed journals or in conference proceedings (N/A for none)

Number of Papers published in non peer-reviewed journals: 0.00

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##### (c) Presentations

Invited lecture "Interfacial chemistry in distributed detection," Department of Homeland Security Workshop on Distributed Detection, Austin, Texas, March 16th, 2009.

Plenary lecture "Designing surface chemistry for protein and cell membrane microarrays," 32nd Senior Technical Meeting, ACS Puerto Rico Chapter, Rincon, November 21st, 2008.

Number of Presentations: 2.00

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##### Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

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##### Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

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##### (d) Manuscripts

Number of Manuscripts: 0.00

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Number of Inventions:

Graduate Students

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
Matthew Goertz	1.00
<b>FTE Equivalent:</b>	<b>1.00</b>
<b>Total Number:</b>	<b>1</b>

Names of Post Doctorates

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
Lei Shen	1.00
<b>FTE Equivalent:</b>	<b>1.00</b>
<b>Total Number:</b>	<b>1</b>

Names of Faculty Supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
<b>FTE Equivalent:</b>	
<b>Total Number:</b>	

Names of Under Graduate students supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
<b>FTE Equivalent:</b>	
<b>Total Number:</b>	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: .....	0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....	0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....	0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale): .....	0.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering: .....	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense .....	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: .....	0.00

Names of Personnel receiving masters degrees

<u>NAME</u>
<b>Total Number:</b>

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**Names of personnel receiving PhDs**

NAME

Matthew Goertz

**Total Number:**

1

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**Names of other research staff**

NAME

PERCENT SUPPORTED

**FTE Equivalent:**

**Total Number:**

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**Sub Contractors (DD882)**

**Inventions (DD882)**

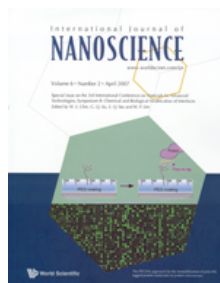
# Controlling Protein Conformation & Activities on Block-Copolymer Nanopatterns

\$\$ Army Research Office

PIs: Xiaoyang Zhu, Tim P. Lodge

Dept. of Chemistry,  
Dept. of Chem. Engin. & Mater. Sci.  
University of Minnesota, Minneapolis

Funding period: 07/01/08-06/30/09

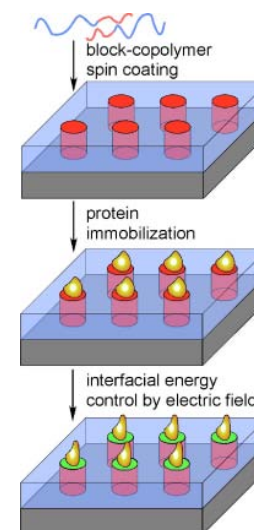


Xiaoyang Zhu has moved to the University of Texas at Austin, effective July 1<sup>st</sup> 2009. The University of Minnesota has relinquished the rights to W911NF0810287. The PI (zhu) has submitted the proposal from UT-Austin for the transfer of the remainder of the grant.

1

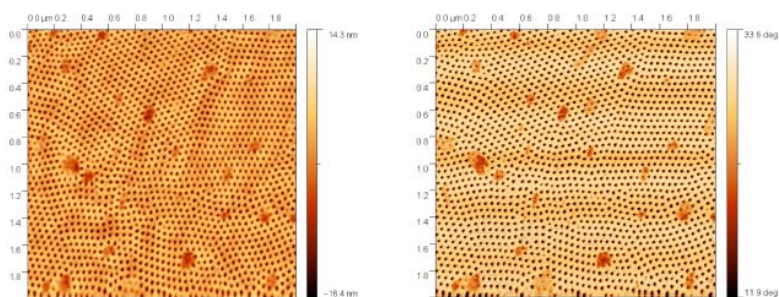
## Research aims

- To use block copolymer thin films in the vertical cylinder phase to create ordered patterns for protein immobilization.
- To measure the activity of model enzymes and the folding efficiency of model proteins immobilized on the nanoscopically patterned surfaces.
- To explore active control of local chemical environment and thus the conformation/activity of immobilized protein molecules, based on electrowetting.



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## Growing vertically oriented cylinder phase

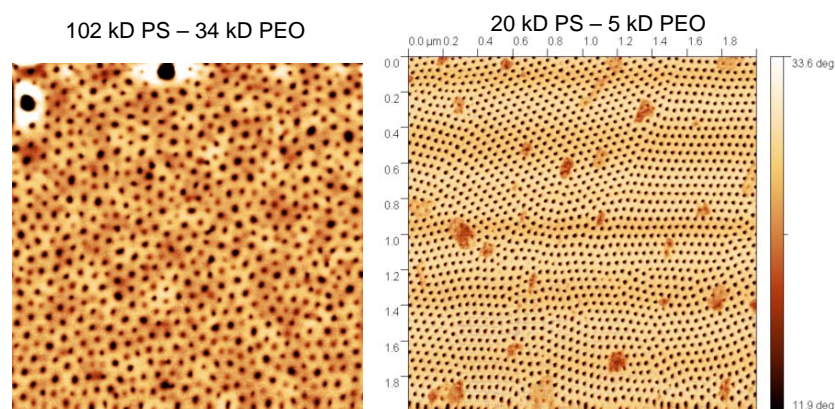


PEO cylinders in PS matrix  
20 kD PS – 5 kD PEO

Atomic Force Microscopy (AFM) images (phase – right; height – left) of PS-PEO (20-5) spin-coated on a silicon wafer from 2% (w/w) in benzene. Solvent annealed for ~24 hours. R.H. ~90-100% Images Taken 12-15-2008

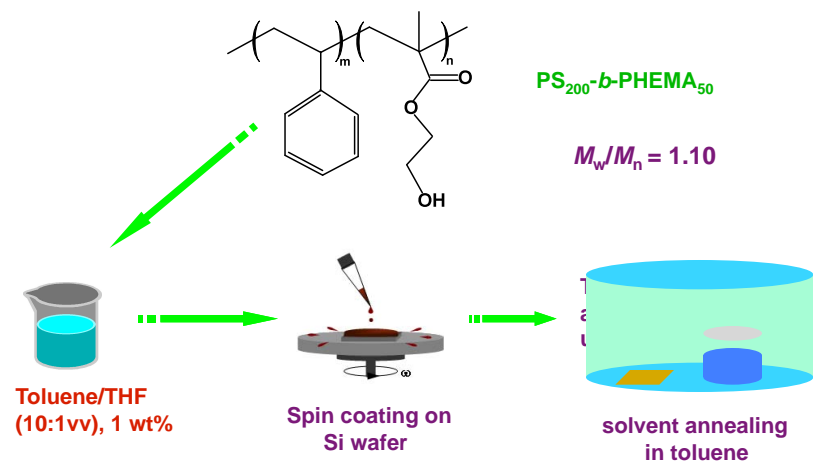
3

## Controlling domain dimensions



4

## A new block co-polymer system: PS-PHEMA

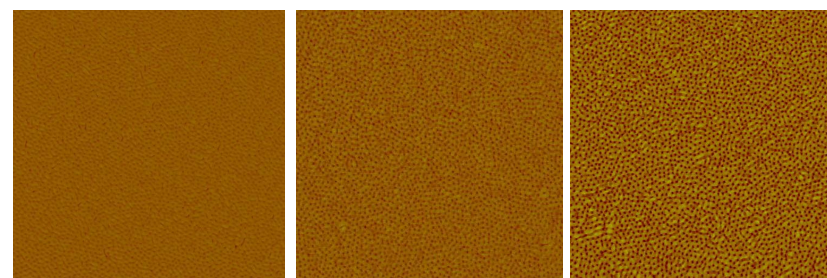


PHEMA is hydrophilic and bio-compatible

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## Ordered phases from PS-PHEMA

AFM images (1.8  $\mu\text{m} \times 1.8 \mu\text{m}$ , phase) as a function of solvent annealing time



0

2 hours

24 hours

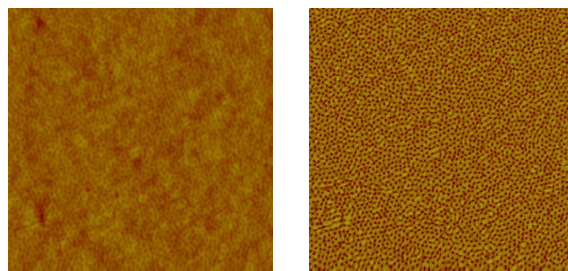
Solvent annealing in Toluene/ethanol  
 PHEMA cylinders in PS matrix

6

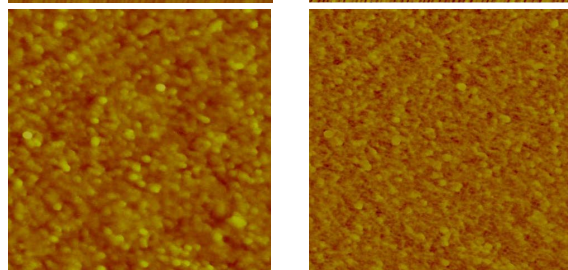
## Passive protein adsorption on the nanopattern

AFM images (1.8  $\mu\text{m} \times 1.8 \mu\text{m}$ )

Without protein



With protein (BSA):  
 Immersed in PBS (6  $\mu\text{g/mL}$ ) for 2h  
 and rinsed with water.

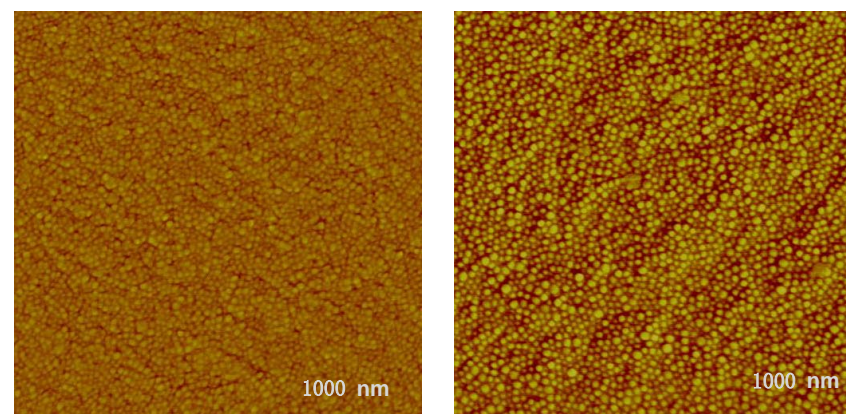


height

phase

7

## PS-*b*-PHEMA thin films: a new morphology



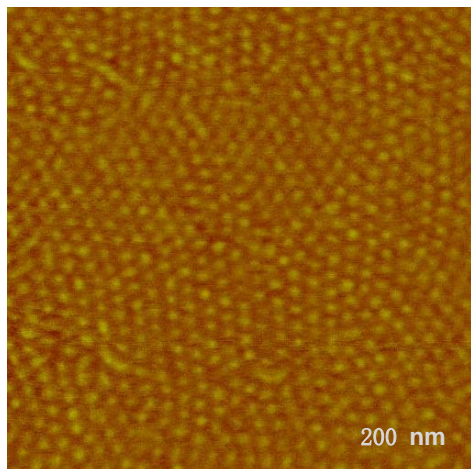
6 h

24 h

THF solution, annealed in toluene/water vapor. The majority PS block segregates on the surface with micell structures.

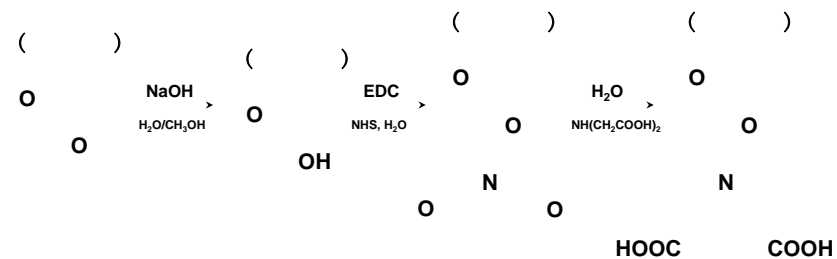
8

## PS-PMMA films:



9

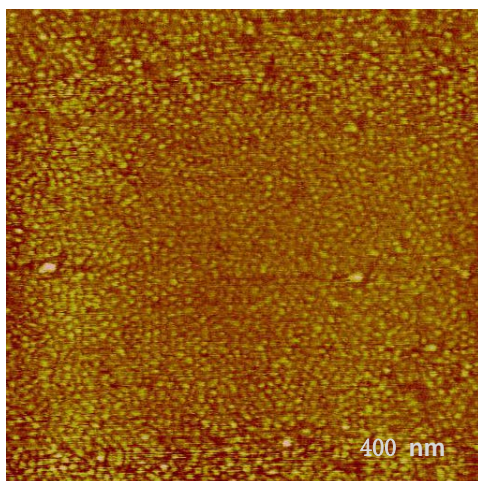
## Chemical modification of the PMMA cylinders



For the chelation of  $\text{Cu}^{2+}$  and the immobilization of poly-His tagged protein molecules

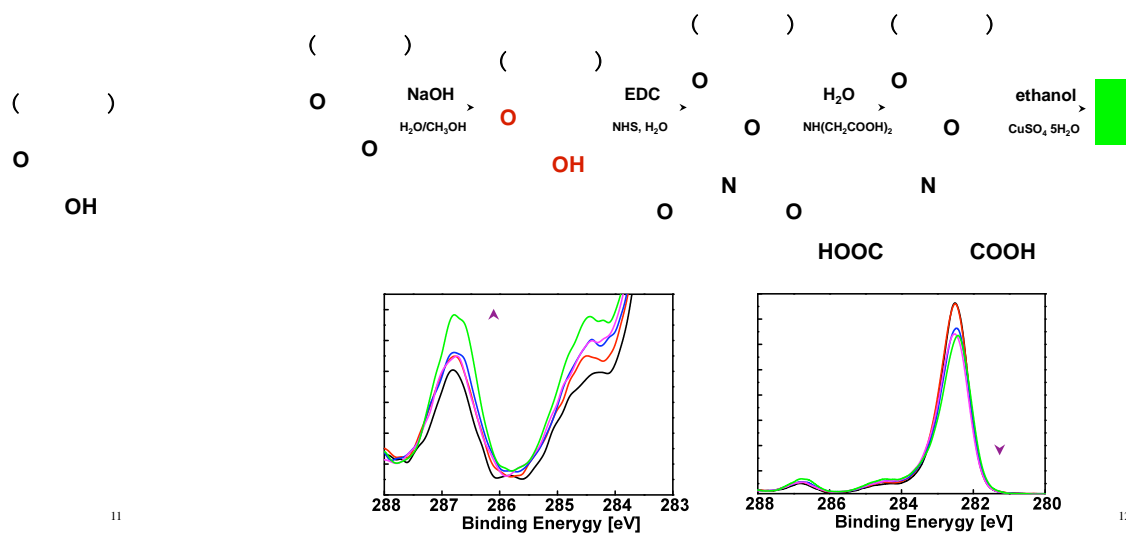
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## PS-b-PMMA film after hydrolysis by NaOH

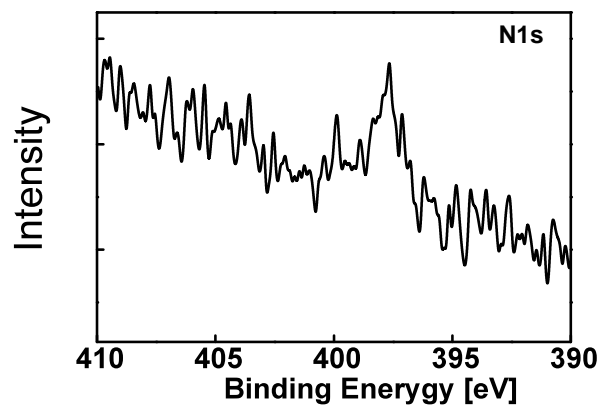


11

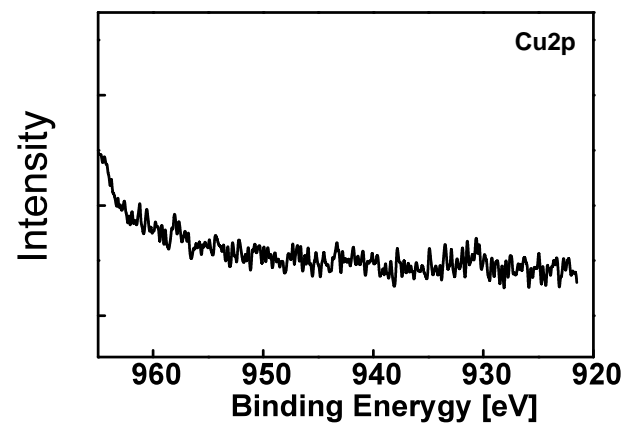
## X-ray photoelectron spectroscopy (XPS)



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## Next steps

- Immobilize model protein molecules on the block-copolymer nanopatterns. Measure protein activity and binding kinetics as a function of pattern dimension and average surface energy.
- Explore protein folding efficiency on nano patterns, as a function of local chemical environment.

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